UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: January 17, 2012

SUBJECT: **N-Butyl-1,2-Benzisothiazolin-3-One (BBIT):** DERs of Metabolism and Pharmacokinetics Studies Submitted for the Existing Registration of Vanquish® Technical

PC Code(s): 098951	DP Barcode(s)/No(s): D386070
Decision No.: 442760	Registration No(s).: 1258-1267
Petition No(s).: NA	Regulatory Action: Action Code 570
Risk Assess Type: Single Chemical	Case No(s).: None
TXR No.: 1,003,231	CAS No(s): 4299-07-4
MRID No(s).: 48262201; 48262203; 48349201	40 CFR: N/A

FROM: Jenny J. Tao, Senior Toxicologist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD) (7510P)

TO: Marshall Swindell, Product Manager
Dennis Edwards, Chief
Regulatory Management Branch I
AD

THRU: Michelle M. Centra, Pharmacologist (peer reviewer) Michelle M. Centra

And

Nader Elkassabany, Ph.D., Chief Nader Elkassabany
RASSB
AD

Rec'd in RRC 2/1/2012 aw

The registrant, Arch Chemical, Inc., submitted the following mammalian toxicity studies and stated in the transmittal letter that "These studies were originally performed for the European Biocides Products Directive and is being sent to the Agency in further support of our registrations containing N-butyl-1,2-benzisothiazolin-3-one." Vanquish® Technical, a manufacturing use product (MUP), is an existing conditional registration and contains 99.2% of N-butyl-1,2-benzisothiazolin-3-one as the active ingredient.

This memo includes executive summary of the reviews and DERs of the submitted studies. The submitted toxicity studies include:

MRID 48262201: A Repeat dose metabolism and pharmacokinetics (tissue distribution time course) study of N-butyl benzisothiazolin-3-one in rats

MRID 48262203: Metabolite identification study of N-butyl benzisothiazolin-3-one in rats

MRID 48349201: Absorption, distribution, metabolism, and excretion study of N-butyl benzisothiazolin-3-one in rats

(1) Metabolism Study – Rats

Smith, A.D. (2006). N-Butyl Benzisothiazolin-3-One: Absorption, Distribution, Metabolism and Excretion in the Rat. Syngenta Limited (CTL). Laboratory Project ID: CO04375r1, Report ID: UR0868-REG, October 6, 2006. MRID 48349201. Unpublished.

Wake, A. (2006). N-Butyl Benzisothiazolin-3-One: Metabolite Identification Work. Syngenta Limited (CTL). Laboratory Project ID: CO04375r1, Report ID: UR0869-REG, November 23, 2006. MRID 48262203. Unpublished.

EXECUTIVE SUMMARY: This Data Evaluation Record summarizes a metabolism study (MRID48349201) and metabolite identification study (MRID48262203) with N-butylbenzisothiazolin-3-one.

In a metabolism study (MRID 48349201), ^{14}C-radiolabelled N-butyl benzisothiazolin-3-one (>98% a.i., batch # SEL/1653), was administered to male Han Wistar rats (4/dose for pharmacokinetic study, 2/dose for expired air study, 4/dose for excretion balance study, and 20/dose for tissue depletion study) via a single oral dose at levels of 5 or 300 mg [^{14}C]-BBIT/kg. For the metabolite identification study (MRID48262203), 14 repeated doses of 5 mg [^{14}C]-BBIT/kg were also given.

Peak concentrations of [^{14}C]-BBIT were measured in blood of rats 1 hour and 4 hours, respectively, following oral administration of the 5 mg/kg or 300 mg/kg doses. Blood concentrations declined quickly and were below the limits of detection within 48 hours for both doses. The greatest concentrations of radioactivity were seen in the bladder and GI tract within 1 hour and 4 hours post dosing and declined rapidly within 48 hours for both doses. Residual radioactivity 3 days post-dosing, accounting for 0.3% of the total administered dose, was seen in the lymph nodes, abdominal fat, pancreas, skin, residual carcass, liver, and kidney. The majority of the administered doses were excreted in the urine and feces, negligible amounts were detected in expired air. Overall, tissue distribution and elimination profiles for the 5 mg/kg and 30 mg/kg dose were similar.

Metabolism of [^{14}C]-BBIT following oral administration of 5 mg [^{14}C]-BBIT/kg or 300 mg [^{14}C]-BBIT/kg in rats is similar and essentially complete with only small amounts of un-metabolized test substance (<0.5% of administered doses) being detected in the urine and feces. Only trace amounts of metabolites were detected in expired air. The predominant urinary metabolites of [^{14}C]-BBIT were identified as a methyl sulfoxide derivative (Metabolite 5, 26-27% of dose), an S-glucoronide metabolite (Metabolite 7, 23-24% of dose), and a methyl sulfoxide metabolite of BBIT with carbonyl substitution (Metabolite 11, 11-18% of dose). These metabolites were also detected in the feces, although at lower and more variable concentrations. Based on the identified metabolites of BBIT, its metabolism is thought to occur via two major routes. Route one involves opening of the isothiazoline ring followed by conjugation of the sulfur atom with glucuronic acid. Route two involves direct oxidation and methylation of the sulfur atom in the isothiazoline rings forming the methyl sulphoxide which then undergoes further oxidation of the N-alkyl side chain and/or N-demethylation.

For the 14-day repeated 5 mg/kg doses study (MRID48262203), urinary metabolite profiles were qualitatively similar between samples collected over 24 hours after the first and final doses and consistent with the single 5 mg/kg dose test results. Same similarity was found for fecal metabolite profiles for samples collected over 24 hours after the first and final doses; however, higher amount of parent material and metabolite 6 were observed following repeated dosing.

The metabolism study (MRID48349201) in the rat is classified as acceptable and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

The metabolism study (MRID 48262203) in the rat is classified acceptable and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

(2) Metabolism and Pharmacokinetics (Tissue Distribution Time Course Study) – Rats

Smith, A.D. (2006) N-butyl benzisothiazolin-3-one: Repeat dose study in the rat (metabolism and pharmacokinetics). Syngenta Limited (CTL), Cheshire, UK., Laboratory Project ID: Syngenta Number CO04375rl,Report ID: UR0884-REG, October 6, 2006. MRID 48262201. Unpublished.

EXECUTIVE SUMMARY: In a tissue distribution time course study (MRID 48262201), [^{14}C]-N-butyl benzisothiazolin-3-one (radiolabelled at the benzene ring, > 98% radiochemical purity, batch # SEL/1653) was administered to 30 male Han Wistar rats, by gavage, at a nominal dose level of 5 mg/kg/day for up to 14 days (equivalent nominal radioactive dose of 27.03 μCi/kg/day). Select tissues were measured for radioactive content for up to 60 days after the last administered dose.

The majority of the administered radioactivity was excreted in the urine and feces. Approximately 87% of the first administered dose and 92% of the 14th administered daily dose were excreted in the urine within 24 hours of dosing, while approximately 15% of the first and 14th doses were excreted in the feces within 24 hours post-dosing.

The tissue distribution of radioactivity following oral dosing with [^{14}C]-N-butyl benzisothiazolin-3-one was widespread; however, the concentrations in individual tissues were all very low. The highest concentrations of radioactivity were found in the G.I. tract plus contents, skin, bladder, residual carcass, liver and kidneys. High concentrations in the G.I. tract and bladder were attributed to the presence of feces and urine in these organs; and high concentrations in the liver and kidneys can be explained because these organs are the major sites of metabolism and excretion for N-butyl benzisothiazolin-3-one. High concentrations in the skin and residual carcass (0.440 and 0.201 μg equivalents/g of tissue, respectively) can be explained by lipophilic deposits in these tissues. After termination of dosing, the concentrations of radioactivity in the majority of tissues declined rapidly and reached background levels within 7 days. Concentrations of radioactivity in the liver, kidneys, skin, and residual carcass were slower to decline but only those in the skin and residual carcass were measurable by 60 days after last dose. The calculated elimination half-lives of N-butyl benzisothiazolin-3-one from the skin and residual carcass were 29.6 and 27.4 days, respectively. There was no evidence to suggest persistence of N-butyl benzisothiazolin-3-one in any of the tissues or organs that were examined.

This tissue distribution time course study in the rat is classified **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a Tier 2 metabolism and pharmacokinetics study [OPPTS 870.7485] in the rat.

DERs of the submitted studies are attached.

Sign-off Date : 01/17/12
DP Barcode No. : D386070

TXR No. : 1,003,231

EPA Reviewer: Jenny J. Tao **Signature:**
Risk Assessment and Science Support Branch
Antimicrobial Division **Date:** 1/17/12

EPA Secondary Reviewer: Michelle M. Centra **Signature:** Michelle M. Centra
Risk Assessment and Science Support Branch
Antimicrobial Division **Date:** 1/19/12

TXR#: 1,003,231

DATA EVALUATION RECORD

STUDY TYPE: Metabolism and pharmacokinetics (tissue distribution time course study) – rat; OPPTS 870.7485 [§85-1)]

PC CODE: 098951 **DP BARCODE:** D386071

TEST MATERIAL (PURITY): [^{14}C]-N-butyl benzisothiazolin-3-one (BBIT) (> 98% radiochemical purity)

SYNONYMS: Vanquish 100; 2-butyl-benzo [d]isothiazol-3 -one; N-butyl-1 ,2-benzisothiazolone; 1 ,2-benzisothiazolin-3-one, 2-butyl; BBIT

CITATION: Smith, A.D. (2006) N-butyl benzisothiazolin-3-one: Repeat dose study in the rat (metabolism and pharmacokinetics). Syngenta Limited (CTL), Cheshire, UK., Laboratory Project ID: Syngenta Number CO04375rl,Report ID: UR0884-REG, October 6, 2006. MRID 48262201. Unpublished.

SPONSOR: Arch Chemicals, Norwalk, CT, USA

EXECUTIVE SUMMARY: In a tissue distribution time course study (MRID 48262201), [^{14}C]-N-butyl benzisothiazolin-3-one (radiolabelled at the benzene ring, > 98% radiochemical purity, batch # SEL/1653) was administered to 30 male Han Wistar rats, by gavage, at a nominal dose level of 5 mg/kg/day for up to 14 days (equivalent nominal radioactive dose of 27.03 µCi/kg/day). Select tissues were measured for radioactive content for up to 60 days after the last administered dose.

The majority of the administered radioactivity was excreted in the urine and feces. Approximately 87% of the first administered dose and 92% of the 14th administered daily dose were excreted in the urine within 24 hours of dosing, while approximately 15% of the first and 14th doses were excreted in the feces within 24 hours post-dosing.

The tissue distribution of radioactivity following oral dosing with [^{14}C]-N-butyl benzisothiazolin-3-one was widespread; however, the concentrations in individual tissues were all very low. The highest concentrations of radioactivity were found in the G.I. tract plus

contents, skin, bladder, residual carcass, liver and kidneys. High concentrations in the G.I. tract and bladder were attributed to the presence of feces and urine in these organs; and high concentrations in the liver and kidneys can be explained because these organs are the major sites of metabolism and excretion for N-butyl benzisothiazolin-3-one. High concentrations in the skin and residual carcass (0.440 and 0.201 µg equivalents/g of tissue, respectively) can be explained by lipophilic deposits in these tissues. After termination of dosing, the concentrations of radioactivity in the majority of tissues declined rapidly and reached background levels within 7 days. Concentrations of radioactivity in the liver, kidneys, skin, and residual carcass were slower to decline but only those in the skin and residual carcass were measurable by 60 days after last dose. The calculated elimination half-lives of N-butyl benzisothiazolin-3-one from the skin and residual carcass were 29.6 and 27.4 days, respectively. There was no evidence to suggest persistence of N-butyl benzisothiazolin-3-one in any of the tissues or organs that were examined.

This tissue distribution time course study in the rat is classified **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a Tier 2 metabolism and pharmacokinetics study [OPPTS 870.7485] in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

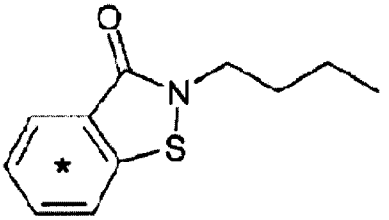
I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test compound:**

Radiolabelled test material:	[^{14}C]-N-butyl benzisothiazolin-3-one
Radiochemical purity:	> 98% determined by TLC
Specific activity:	1070.3GBq/mMole; 5.140 MBq/mg
Lot/batch #:	SEL/1653
Non-Radiolabelled test material:	N-Butyl-1, 2-Benzisothiazolin-3-one
Description:	Pale brown liquid, technical grade, expiry date May 17, 2007
Lot/batch #:	6182
Purity:	98.9 ± 1% a.i. determined by HPLC
Contaminants:	Not reported
CAS # of TGAI:	4299-07-4

Structure:



* = denotes position of radiolabel

2. **Vehicle and/or positive control:** corn oil, CTL Reference # Y00790/014, Purity not reported

3. **Test animals:**

Species:	Rat
Strain:	Han Wistar (HsdBrlHan: Wistar)
Age/weight at study initiation:	Age not reported, 210–233 g
Source:	Harlan UK Shaw's Farm Blackthorn Bicester Oxon OX6 0TP
Housing:	Up to 3/cage in multiple rat racks
Diet:	Rat and Mouse No. 1 maintenance diet (Special Diets Services) provided *ad libitum*, analyzed for contaminants (specific details not reported)
Water:	By automatic system or in drinking water bottles provided *ad libitum*, analyzed for contaminants (specific details and source of water not reported)
Environmental conditions:	
Temperature:	22 ± 3°C
Humidity:	30–70%
Air changes:	At least 15/hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 7 days

4. Preparation of dosing solutions: Dosing samples, formulated by mixing the appropriate amounts of radiolabelled and unlabelled test material in corn oil, were prepared one day before usage every four days during dosing. Storage details were not reported; however, it was reported that the stability of the test compound in the vehicle was established for an interval longer than its intended use. Each dose formulation was analyzed in triplicate for specific activity of the [^{14}C]- N-butyl benzisothiazolin-3-one and homogeneity using liquid scintillation counting prior to dosing. Homogeneity and stability of the test solutions was considered to be satisfactory.

B. STUDY DESIGN AND METHODS:

1. Group arrangements:

Animals were assigned randomly to the test groups noted in Table 1.

TABLE 1: Dosing groups for a tissue distribution time course study for N-butyl benzisothiazolin-3-one (BBIT) in Han Wistar rats

Test group	Target Dose of labeled material (mg/kg)	Number/sex	Remarks
1	5	3 males	Sacrificed 1 day after the 3rd dose
2	5	3 males	Sacrificed 1 day after the 7th dose
3	5	3 males	Sacrificed 1 day after the 10th dose
4	5	3 males	Sacrificed 1 day after the 14th dose
5	5	3 males	Sacrificed 3 days after the 14th dose
6	5	3 males	Sacrificed 7 days after the 14th dose
7	5	3 males	Sacrificed 10 days after the 14th dose
8	5	3 males	Sacrificed 14 days after the 14th dose
9	5	3 males	Sacrificed 28 days after the 14th dose
10	5	3 males	Urine and feces collected for 24 hrs following 1st and 14th doses; Sacrificed 60 days after the 14th dose

2. Dosing and sample collection:

a. **Pharmacokinetic studies:** A target dose of 5 mg [^{14}C]-N-butyl benzisothiazolin-3-one/kg bw (corresponding to a nominal dose of 27.03 μCi/kg) was administered daily by gavage to thirty male rats at a dose rate of 2 mL/kg for up to 14 days. Each rat was weighed daily and the dosing volume was adjusted accordingly for each rat. Three rats were housed in glass metabolism chambers for 24 hours after dosing on days 1 and 14 only. Collection vessels were cooled by solid carbon dioxide. Urine and feces were collected, after the first and last doses, with undefined number of collections during the 24-hour post-dosing period. At the end of 24 hours, each rat was removed from the metabolism cages and returned to the stock cages. The metabolism cages were washed

with a water:ethanol (50:50) solution and the washings were collected separately. Expired air was not collected.

Rats were sacrificed by exsanguination in groups of three at the following time points: 1 day after the 3rd, 7th, and 10th doses and 1, 3, 7, 10, 14, 28 and 60 days after the final (14th) dose. Terminal blood, organ, and tissue samples were collected from each rat and analyzed for radioactivity. The following whole tissues were analyzed for radioactivity: adrenals, brain, heart, kidneys, liver, thyroid, salivary glands, eyes, urinary bladder, gastro intestinal tract (plus contents), lungs, prostate, pancreas, spleen, thymus, testes, pituitary, and epididymis. In addition, samples were collected from the following tissues: bone (femur), fat (abdominal), skin, muscle, lymph nodes (mesenteric), sciatic nerve, and spinal cord. Residual carcasses were also retained for analysis.

Samples were prepared for radioactivity analysis (liquid scintillation counting) in the following manner:

- Feces were homogenized to a paste using small amounts of water and then oxidized using a PackardTri-Carb 307 oxidizer.
- The gastrointestinal tract and its contents were homogenized and then oxidized using a PackardTri-Carb 307 oxidizer.
- Blood and tissues were solubilized in Soluene tissue digestant.
- Residual carcasses were digested using an aqueous sodium hydroxide, ethanol, and Triton X-405 solution.
- Urine, cage wash, plasma and other solutions (including solvent extracts and tissue digests) were diluted with scintillant.

b. Metabolite characterization studies: Metabolite characterization studies were not conducted.

3. Statistics:

Radioactivity was expressed as the percentage of the dose administered for excreta and as µg equivalents of BBIT/g of tissue for tissue concentrations. Percent recovery in the urine, feces and cage wash were expressed as group means with standard deviations. To determine total urinary excretion, the amount of radioactivity present in the cage wash was attributed to urinary excretion. Tissue concentrations were reported individually and as group means with standard deviations. Total absorption after oral administration and tissue-to-blood ratios of radioactivity were not calculated.

The computerized data acquisition system Debra 5.4.10.51 was used to collect and calculate total radioactivity in all samples. The limit of detection (LOD) used for radioactivity in each sample was twice the scintillation counter background rate determined by reagent blanks for each counting batch. When measurements were below the LOD, the computer program automatically recorded the measurement as twice the background rate (the LOD) for the purposes of calculating group mean tissue concentrations. However, the individual results were still reported as <LOD in the study report. Standard deviations were not reported for parameters measured <LOD. Half-lives of elimination were calculated for skin and residual

carcass using the computer program WinNonlin V5. The reviewer considers the analyses used to be appropriate.

II. RESULTS

A. PHARMACOKINETIC STUDIES:

1. **Preliminary experiment:** This study did not include a preliminary experiment.

2. **Absorption:** Absorption was not calculated in this study. The study report stated that following 14 repeated daily oral dose of 5 mg [^{14}C]-BBIT/kd/day, the test substance "was extensively absorbed".

3. **Tissue distribution:** The tissue distribution of measured radioactivity is summarized in Table 2 for each experimental group.

   Oral dose: As shown in Table 2, the tissue distribution of radioactivity following oral dosing with [^{14}C]-N-butyl benzisothiazolin-3-one was widespread; however the concentrations in individual tissues were all very low. The highest concentrations of radioactivity were found in the gastrointestinal (GI) tract plus contents, skin, bladder, residual carcass, liver, and kidneys. The concentrations in these tissues during the dosing phase were all higher than the concentrations measured in plasma. Concentrations in the eyes, adrenal glands, and lymph nodes were slightly higher than in the plasma during the dosing phase. None of the other tissues had concentrations that were consistently higher than plasma throughout the dosing phase; however, some had concentrations that were transiently higher than in plasma (blood, bone, epididymis, prostate, salivary gland, spleen, testes, and thyroid).

   The skin and liver were the only tissues exhibiting a steady increase in radioactivity throughout the dosing phase. Measured concentrations were 0.440 and 0.167 µg equivalents/g of tissue, respectively, after 14 days of dosing. After daily dosing was terminated, radioactivity concentration in all tissues decreased throughout the remainder of the study, with the concentration in most tissues falling below the limits of detection within 7 days. The liver, kidneys, skin, and residual carcass exhibited slower decreases in radioactivity compared with other tissues. Radioactivity concentration 60 days after the final administered dose was highest in the skin (0.093 µg equivalents/g of tissue) followed by residual carcass (0.041 µg equivalents/g of tissue). The calculated elimination half-lives of these tissues were 29.6 and 27.4 days, respectively. Elimination half-lives were not calculated for any other tissues due to the very low concentrations or absence of measureable residues.

TABLE 2: Mean concentrations of total radioactivity in male rat tissues/organs following oral administration of 5 mg [^{14}C]-N-butyl benzisothiazolin-3-one [a,b,c]

Tissue/organ	µg Equivalents of BBIT/g of tissue [d]									
	Day 4 (3 days of dosing)	Day 8 (7 days of dosing)	Day 11 (10 days of dosing)	Day 15 (14 days of dosing)	Day 17 (3 days after last dose)	Day 21 (7 days after last dose)	Day 24 (10 days after last dose)	Day 28 (14 days after last dose)	Day 42 (28 days after last dose)	Day 74 (60 days after last dose)
Abdominal Fat	<0.024	0.016 ± 0.002	<0.016	<0.020	<0.059	<0.014	<0.014	<0.015	<0.027	<0.014
Adrenals	0.051 ± 0.018	0.051 ± 0.011	0.045 ± 0.01	0.086 ± 0.03	<0.153	<0.052	<0.048	<0.055	<0.052	<0.031
Bladder	0.328 ± 0.339	0.269 ± 0.322	0.213 ± 0.144	0.086 ± 0.057	<0.223	<0.104	<0.090	<0.070	<0.028	<0.016
Blood	<0.056	0.049 ± 0.006	<0.048	<0.069	<0.087	<0.094	<0.048	<0.046	<0.044	<0.046
Bone	<0.05	0.035 ± 0.004	<0.034	0.027 ± 0.02	<0.032	<0.029	<0.028	<0.017	<0.008	<0.009
Brain	0.017 ± 0.004	0.016 ± 0.001	0.016 ± 0.003	0.024 ± 0.014	<0.023	<0.010	<0.015	<0.015	<0.008	<0.012
Epididymis	0.042 ± 0.018	0.033 ± 0.006	0.030 ± 0.004	0.042 ± 0.024	0.044 ± 0.03	0.022 ± 0.01	<0.012	<0.010	<0.012	<0.014
Eyes	0.043 ± 0.009	0.052 ± 0.012	0.044 ± 0.005	0.05 ± 0.022	<0.032	<0.022	<0.023	<0.024	<0.015	<0.001
G.I. tract + contents	1.238 ± 0.580	1.224 ± 0.397	1.714 ± 0.942	1.315 ± 0.448	0.409 ± 0.266	0.519 ± 0.234	0.139 ± 0.151	0.054 ± 0.015	<0.017	<0.012
Heart	0.034 ± 0.018	0.024 ± 0.002	0.020 ± 0.009	<0.034	<0.012	<0.016	<0.019	<0.012	<0.008	<0.013
Kidneys	0.097 ± 0.058	0.088 ± 0.015	0.085 ± 0.015	0.146 ± 0.077	0.098 ± 0.08	0.058 ± 0.032	0.029 ± 0.016	0.027 ± 0.010	0.011 ± 0.000	<0.011
Liver	0.111 ± 0.036	0.124 ± 0.004	0.134 ± 0.029	0.167 ± 0.055	0.111 ± 0.038	0.066 ± 0.014	0.035 ± 0.018	0.028 ± 0.004	<0.013	<0.010
Lungs	0.028 ± 0.014	0.031 ± 0.003	0.030 ± 0.006	<0.042	<0.028	<0.020	<0.022	<0.013	<0.010	<0.013
Lymph nodes	0.075 ± 0.074	0.038 ± 0.008	0.034 ± 0.004	0.058 ± 0.03	0.066 ± 0.064	<0.019	<0.014	<0.016	<0.033	<0.014

TABLE 2: Mean concentrations of total radioactivity in male rat tissues/organs following oral administration of 5 mg [^{14}C]-N-butyl benzisothiazolin-3-one [a,b,c]

Tissue/organ	µg Equivalents of BBIT/g of tissue [d]									
	Day 4 (3 days of dosing)	Day 8 (7 days of dosing)	Day 11 (10 days of dosing)	Day 15 (14 days of dosing)	Day 17 (3 days after last dose)	Day 21 (7 days after last dose)	Day 24 (10 days after last dose)	Day 28 (14 days after last dose)	Day 42 (28 days after last dose)	Day 74 (60 days after last dose)
Muscle	0.035 ± 0.017	0.023 ± 0.003	0.028 ± 0.014	0.037 ± 0.029	<0.027	<0.027	<0.012	<0.014	<0.011	<0.012
Pancreas	<0.048	<0.040	<0.035	0.041 ± 0.027	0.118 ± 0.153	<0.021	<0.018	<0.021	<0.026	<0.019
Pituitary gland	<0.725	<0.574	<1.206	<0.253	<0.266	<0.404	<0.49	<0.411	<0.432	<0.125
Plasma	0.040 ± 0.018	0.033 ± 0.002	0.025 ± 0.004	0.052 ± 0.037	0.028 ± 0.015	<0.021	<0.009	<0.008	<0.008	<0.011
Prostate	0.054 ± 0.017	<0.035	0.071 ± 0.035	<0.097	0.053 ± 0.035	<0.026	<0.02	<0.018	<0.015	<0.023
Residual carcass	0.098 ± 0.018	0.0120 ± 0.017	0.12 ± 0.017	0.186 ± 0.028	0.17 ± 0.039	0.159 ± 0.056	0.139 ± 0.029	0.145 ± 0.024	0.091 ± 0.010	0.041 ± 0.002
Salivary gland	0.037 ± 0.012	0.034 ± 0.007	0.032 ± 0.006	<0.052	0.033 ± 0.023	<0.022	<0.015	<0.014	<0.009	<0.014
Sciatic nerve	<0.072	<0.056	<0.065	<0.114	<0.092	<0.161	<0.055	<0.097	<0.064	<0.042
Skin	0.102 ± 0.009	0.246 ± 0.084	0.338 ± 0.151	0.440 ± 0.051	0.426 ± 0.233	0.401 ± 0.189	0.181 ± 0.092	0.226 ± 0.076	0.190 ± 0.050	0.093 ± 0.015
Spinal cord	<0.020	<0.029	<0.017	<0.040	<0.018	<0.030	<0.022	<0.034	<0.015	<0.015
Spleen	0.035 ± 0.015	0.038 ± 0.013	0.036 ± 0.006	<0.036	0.029 ± 0.019	<0.022	<0.01	<0.014	<0.011	<0.014
Testes	0.041 ± 0.012	0.032 ± 0.004	0.033 ± 0.003	0.054 ± 0.041	<0.028	<0.020	<0.013	<0.018	<0.010	<0.012
Thymus	0.034 ± 0.018	0.027 ± 0.004	0.026 ± 0.004	<0.041	0.021 ± 0.021	<0.020	<0.017	<0.018	<0.011	<0.017
Thyroid	<0.111	0.352 ± 0.182	<0.154	<0.291	<0.287	<0.235	<0.231	<0.224	<0.152	<0.072

[a] Data obtained from pages 17, 24–25 and 43–52 in the study report.
[b] n=3
[c] < denotes below the limit of detection; standard deviation not reported
[d] µg BBIT eq/g = (Mean d.p.m/ g in sample) ÷ [specific activity (d.p.m./ µg)]

4. **Excretion:** The radioactivity measured in the excreta of male rats following a single dose and the 14th daily dose of [^{14}C]-N-butyl benzisothiazolin-3-one is shown in Table 3.

TABLE 3: Recovery of radioactivity in excreta of male rats following oral administration of 5mg/kg [^{14}C]-N-butyl benzisothiazolin-3-one [a,b]

	Percent of radioactive dose recovered [c]	
	0–24 hrs after 1st dose	0–24 hrs after 14th daily dose
Cage wash	11.75 ± 1.77	6.04 ± 1.28
Urine	75.30 ± 6.11	86.18 ± 2.09
Total Urine [d]	87.05	92.22
Feces	15.17 ± 6.43	14.63 ± 1.06
Total	102.22 ± 12.20	106.85 ± 0.72

[a] Data obtained from pages 17 and 53 in the study report.
[b] n=3
[c] Percent recovered dose = (Total d.p.m in sample) ÷ (Total d.p.m administered) × 100
[d] Total urine = cage wash + urine

Oral dose: As summarized in Table 3, the urine appears to be the major route of excretion for N-butyl benzisothiazolin-3-one with approximately 87% of the first administered dose being excreted within 24 hours of dosing (including the cage wash). Urinary excretion was higher following the 14th dose, with approximately 92% of the administered dose excreted in the urine within 24 hours of dosing. Excretion in the feces was approximately 15% of the oral dose following both the first and 14th doses for the 24-hour post-dosing period.

B. METABOLITE CHARACTERIZATION STUDIES: Not applicable

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: [^{14}C]-N-butyl benzisothiazolin-3-one was rapidly and extensively absorbed and widely distributed to tissues following daily oral administration of 5 mg/kg to male rats for up to 14 days. Tissue radioactivity concentrations during dosing represented only a small fraction of the administered dose as the majority of the administered dose was excreted in the urine and feces.

After dosing was terminated, the residual radioactivity levels declined rapidly in the vast majority of tissues and reached background levels within three to seven days. However, residual radioactivity concentrations in tissues associated with lipophilic deposits (skin and residual carcass) declined at a slower rate and required up to 60 days to approach background levels. However, no tissues showed evidence of persistence.

Radioactivity measured in the urine following the first and 14th doses indicates that approximately 85% of the administered dose was absorbed. The apparent increase in urinary residues from the first to the 14th dose was attributed to some radioactivity being excreted from previous doses. The highest tissue residue concentration was associated with the G.I. tract and its contents, which was linked with the presence of fecal matter. Likewise, the radioactivity concentrations measured in the bladder were attributed to the presence of urine. Other tissues containing relatively high concentrations of radioactivity were the skin and residual carcass, which were likely associated with lipophilic deposits. Residue concentrations in other tissues were variable, with the exception of the liver and kidneys. Radioactivity measurements were consistently higher in these organs compared with plasma likely because these are the primary organs for metabolism and excretion of N-butyl benzisothiazolin-3-one. Tissues with residue concentrations that were slightly or transiently elevated above plasma concentrations were not considered to be of consequence.

B. REVIEWER COMMENTS: The reviewer agrees with the investigator's conclusions. The urinary excretion data indicates that N-butyl benzisothiazolin-3-one is rapidly and highly absorbed following oral administration. Tissue distribution measurements indicate that the skin and lipophilic reservoirs in the residual carcass are the areas of highest concentration of N-butyl benzisothiazolin-3-one following absorption. The concentration of radioactivity in the skin demonstrated an increasing trend during daily dosing which may have continued if the dosing phase had been extended. The concentration of radioactivity in the skin following the 14th daily dose was approximately 8.5 times higher than the concentration in the plasma. The half-life of elimination from the skin was calculated to be 29.6 days; therefore, the evidence does not suggest long term persistence of N-butyl benzisothiazolin-3-one in the skin. Furthermore, there was no evidence to suggest persistence of this compound in any of the other tissues or organs that were examined.

The study had several minor deficiencies noted below; however, these do not change the conclusions of this review. One of the minor deficiencies with this study is that the investigator did not explicitly report the route of administration as gavage. However, the route was reported to be oral and a dosing syringe was mentioned. The mention of a dosing syringe along with the fact that corn oil was used as the vehicle suggests that the route of administration was gavage. Another minor deficiency of this study is that the justification for not collecting expired air during the excretion portion of this study was not included. However, this is not necessarily important if the main purpose of this study was to define the tissue distribution of N-butyl benzisothiazolin-3-one and the excreta were measured only to confirm adequate absorption.

This study meets the requirements for a Tier 2 metabolism and pharmacokinetics study under the OPPTS 870.7485 guideline. Specifically, it is a tissue distribution time course study. It does not meet the requirements for a Tier 1 metabolism and pharmacokinetics study under the OPPTS 870.7485 guideline.

C. **STUDY DEFICIENCIES:** The following minor deficiencies are noted; however, these do not affect interpretation of the results, nor do they change the conclusions of study.

- Rationale for the choice of vehicle not included
- Impurities in test substance not reported
- Animals age at study initiation not reported
- Route of administration not explicitly reported as gavage
- Justification for not collecting expired air during the study not included
- Quantity of radioactivity in the excreta not reported (only reported as percent of administered dose)
- Tissue distribution of radioactivity only reported as μg equivalents/g of tissue and not also as the percent of administered dose

D. STUDY CLASSIFICATION: This tissue distribution time course study in the rat is classified **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a Tier 2 metabolism and pharmacokinetics study [OPPTS 870.7485] in the rat.

Sign-off Date : 01/17/12
DP Barcode No. : D386070

TXR No. : 1,003,231

EPA Reviewer: Jenny J. Tao
Risk Assessment and Science Support Branch
Antimicrobial Division

Signature: [signature]
Date: 1/17/12

EPA Secondary Reviewer: Michelle M. Centra
Risk Assessment and Science Support Branch
Antimicrobial Division

Signature: Michelle M. Centra
Date: 1/19/12

TXR#: 1,003,231

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS 870.7485 [§85-1)]; OECD 417.

PC CODE: 098951 **DP BARCODE:** D386071

TEST MATERIAL (PURITY): N-butyl benzisothiazolin-3-one (>98%)

SYNONYMS: 2-butyl benzisothiazolin-3-one, 2-butyl-1,2-benzisothiazothiazolin-3-one, butyl-BIT, Vanquish 100, BBIT, N-BBIT

CITATIONS: Smith, A.D. (2006). N-Butyl Benzisothiazolin-3-One: Absorption, Distribution, Metabolism and Excretion in the Rat. Syngenta Limited (CTL). Laboratory Project ID: CO04375r1, Report ID: UR0868-REG, October 6, 2006. MRID 48349201. Unpublished.

Wake, A. (2006). N-Butyl Benzisothiazolin-3-One: Metabolite Identification Work. Syngenta Limited (CTL). Laboratory Project ID: CO04375r1, Report ID: UR0869-REG, November 23, 2006. MRID 48262203. Unpublished.

SPONSOR: Arch Chemicals/ARCH Biocides, Norwalk, CT.

EXECUTIVE SUMMARY: This Data Evaluation Record summarizes a metabolism study (MRID48349201) and metabolite identification study (MRID48262203) with N-butylbenzisothiazolin-3-one.

In a metabolism study (MRID 48349201), ^{14}C-radiolabelled N-butyl benzisothiazolin-3-one (>98% a.i., batch # SEL/1653), was administered to male Han Wistar rats (4/dose for pharmacokinetic study, 2/dose for expired air study, 4/dose for excretion balance study, and 20/dose for tissue depletion study) via a single oral dose at levels of 5 or 300 mg [^{14}C]-BBIT/kg. For the metabolite identification study (MRID48262203), 14 repeated doses of 5 mg [^{14}C]-BBIT/kg were also given.

Peak concentrations of [^{14}C]-BBIT were measured in blood of rats 1 hour and 4 hours, respectively, following oral administration of the 5 mg/kg or 300 mg/kg doses. Blood

concentrations declined quickly and were below the limits of detection within 48 hours for both doses. The greatest concentrations of radioactivity were seen in the bladder and GI tract within 1 hour and 4 hours post dosing and declined rapidly within 48 hours for both doses. Residual radioactivity 3 days post-dosing, accounting for 0.3% of the total administered dose, was seen in the lymph nodes, abdominal fat, pancreas, skin, residual carcass, liver, and kidney. The majority of the administered doses were excreted in the urine and feces, negligible amounts were detected in expired air. Overall, tissue distribution and elimination profiles for the 5 mg/kg and 30 mg/kg dose were similar.

Metabolism of [^{14}C]-BBIT following oral administration of 5 mg [^{14}C]-BBIT/kg or 300 mg [^{14}C]-BBIT/kg in rats is similar and essentially complete with only small amounts of un-metabolized test substance (<0.5% of administered doses) being detected in the urine and feces. Only trace amounts of metabolites were detected in expired air. The predominant urinary metabolites of [^{14}C]-BBIT were identified as a methyl sulfoxide derivative (Metabolite 5, 26-27% of dose), an S-glucoronide metabolite (Metabolite 7, 23-24% of dose), and a methyl sulfoxide metabolite of BBIT with carbonyl substitution (Metabolite 11, 11-18% of dose). These metabolites were also detected in the feces, although at lower and more variable concentrations. Based on the identified metabolites of BBIT, its metabolism is thought to occur via two major routes. Route one involves opening of the isothiazoline ring followed by conjugation of the sulfur atom with glucuronic acid. Route two involves direct oxidation and methylation of the sulfur atom in the isothiazoline rings forming the methyl sulphoxide which then undergoes further oxidation of the N-alkyl side chain and/or N-demethylation.

For the 14-day repeated 5 mg/kg doses study (MRID48262203), urinary metabolite profiles were qualitatively similar between samples collected over 24 hours after the first and final doses and consistent with the single 5 mg/kg dose test results. Same similarity was found for fecal metabolite profiles for samples collected over 24 hours after the first and final doses; however, higher amount of parent material and metabolite 6 were observed following repeated dosing.

The metabolism study (MRID48349201) in the rat is classified as acceptable and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

The metabolism study (MRID 48262203) in the rat is classified acceptable and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

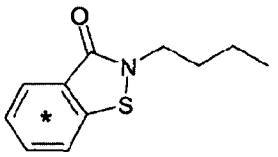
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test compound:**

Radiolabelled test material:	[^{14}C]-BBIT
Radiochemical purity:	>98% determined by Thin Layer Chromatography (TLC)
Specific activity:	5.140 MBq/mg (1070.3 GBq/mMole)
Lot/batch #:	SEL/1653
Non-Radiolabelled test material:	Vanquish 100
Description:	Technical, pale brown liquid
Lot/batch #:	6182
Purity:	98.9 % a.i.
Contaminants:	None reported
CAS # of TGAI:	4299-07-4
Structure:	



* = denotes position of radiolabel

Source: www.chemfinder.com

2. **Vehicle and/or positive control:** Corn oil

3. **Test animals:**

Species:	Rat	
Strain:	Han Wistar HsdBr1Han:Wistar	
Weight at study initiation:	210-273 g	
Source:	Harlan UK Shaw's Farm Blackthorn Bicester Oxon OX6 0TP	
Housing:	Animals were housed up to 5 per cage in multiple rat racks	
Diet:	Rat and mouse No 1 maintenance diet provided ad libitum	
Water:	Supplied by an automatic system or in drinking water bottles ad libitum	
Environmental conditions:	**Temperature:**	22 ± 3°C
	Humidity:	30 to 70%
	Air changes:	At least15 changes/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Animals were housed under experimental conditions for at least 5 days prior to the start of the study.	

4. **Preparation of dosing solutions:** Each dose solution was prepared on the day prior to dosing by combining the required amounts of unlabelled BBIT and [^{14}C]-BBIT and dissolving these test substances in the required amount of corn oil to achieve doses of 5 mg BBIT/kg and 300 mg BBIT/kg. No further details were provided.

5. **Analysis of dosing solutions:** The specific activity of the [^{14}C]–BBIT contained in each dosing solution was determined by liquid scintillation counting immediately after preparation and prior to dosing. This data was used to confirm the homogeneity of the test substance prior to study initiation. The stability of the dosing solutions was measured by comparing the radiochemical purity of the stock [^{14}C]–BBIT used to prepare the solutions and the radiochemical purity of the dosing solutions after dosing. Radiochemical purities were analyzed by thin layer chromatography (TLC).

   Mean calculated administered doses of [^{14}C] –BBIT were 5.216 mg/kg and 284.124 mg/kg which were within 5% of the target doses of 5 mg/kg and 300 mg/kg. The radiochemical purity in the stock solution was 98% while the radiochemical purity in the 5 mg/kg and 300 mg/kg dosing solutions were both greater than 97% confirming that the dosing formulations were stable. The study author stated that that the homogeneity of the dose preparations was acceptable.

B. STUDY DESIGN AND METHODS:

1. **Group arrangements:** Animals were assigned randomly to the test groups noted in Table 1. Testing for group C was repeated due to low radioactivity recoveries in expired carbon dioxide. The percent recoveries from the original study group were not reported. The study report provided data from the repeat group C throughout the entirety of the study (MRID 48349201).

Table 1. Dosing groups for pharmacokinetic/metabolism Studies of [^{14}C]-BBIT[a] in male rats

Test group:	Single Dose of [^{14}C]-BBIT (mg/kg):	Number of rats:	Study Phase:	Used for metabolite identification:
A	5	4	Pharmacokinetics experiment	No
B	300	4	Pharmacokinetics experiment	No
C	5	2	Expired air	Yes
C[b] (repeated)	5	2	Expired air	Yes
D	300	2	Expired air	Yes
E	5	4	Excretion balance	Yes
F	300	4	Excretion balance	Yes
G	5	20	Tissue depletion	No
H	300	20	Tissue depletion	No

[a] Data obtained from pages 17-18 of study report UR0868-REG (MRID 48349201) and page 12 of study report UR0869-REG (MRID 48262203).

[b] Group C experiment was repeated based on low radioactivity recoveries. Data from Group C (repeated) is the only data reported.

2. **Dosing and sample collection:** Rats in Groups A, C, E, and G were given a single oral dose of 5 mg [^{14}C]–BBIT/kg; rats in Groups B, D, F, and H were given a single oral dose of 300 mg [^{14}C]–BBIT/kg. Blood samples were obtained from rats in test Groups A and B, urine, feces, and expired air were collected from test Groups C and D, urine and feces were collected from test Groups E and F, and selected tissue samples were collected from test Groups G and H (Table 1).

   a. **Pharmacokinetic studies:** Blood samples for Groups A and B were obtained from the lateral tail vein and collected into heparinised tubes at 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours following oral administration of [^{14}C]-BBIT. Terminal blood samples were collected 48 hours post-dosing via cardiac puncture. Blood samples were digested or oxidized and analyzed for radioactivity using liquid scintillation counting (LSC).
   b. **Excretion studies**: Excreta from Groups C and D were collected over solid carbon dioxide and stored at approximately -20°C prior to analysis. Volatile metabolites in expired air were collected using a cold trap, cold finger, and charcoal traps. Terminal cage washes were conducted with a 50:50 water:ethanol mixture and the washings were refrigerated prior to analysis. Collection times for Groups C and D samples are summarized in Table 2.

Table 2. Excretion collection times for groups C and D of [^{14}C]-BBIT in male rats[a]

Sample/Trap:	Group:	Collection time (hours):
Nilox columns	D	12, 24
Cold finger	C (rpt)	6, 12, 24
Charcoal	C (rpt) and D	12, 24
Urine	C (rpt)	6, 12, 24, 48
Urine	D	12, 24, 48, 72
Feces	C (rpt)	24, 48
Feces	D	24, 48, 72

[a] Data obtained from page 20 of study report (MRID 48349201).

Feces were homogenized to a paste or oxidized prior to analysis by liquid scintillation counts. Urine and cage washings were diluted prior to analysis. Excreta and terminal cage washings from Groups E and F were collected, stored, and analyzed in a similar manner as described for Groups C and D. Urine was collected at 12 hours and urine and feces at 24, 48, and 72 hours.

Three days post-dosing, terminal blood samples were obtained via cardiac puncture in Groups E, F, G, and H. Sub-groups of 4 animals were sacrificed at 1, 4, 12, 24, and 72 hours for the 5 mg dose and 4, 8, 12, 24, and 48 hours for the 300 mg dose (as determined by absorption study) at time intervals (not reported). The residual carcass and tissues listed in Table 3 were solubilized or digested prior to analysis for radioactive content.

Table 3. Tissue samples collected from sacrificed test animals		
The following whole tissues will be removed:		Together with representative samples of:
Adrenals	Pituitary gland	Bone (femur)
Brain	Urinary bladder	Fat (abdominal)
Heart	Thymus	Muscle
Kidneys	Thyroid	Skin
Liver	Testes	Spinal cord
Lungs	Salivary glands	Lymph nodes
Prostate	Epididymis	Sciatic nerve
Pancreas	Eyes	
Spleen	Gastrointestinal tract and contents	

3. **Metabolite characterization studies:** Identification of metabolites was conducted under MRID48262203. Excreta samples from Groups C, D, E, and F were pooled by group and analyzed qualitatively (to identify the chemical structure of the metabolites) and quantitatively (to determine relative proportions of radiolabelled metabolites). Urine samples were analyzed directly while fecal samples were extracted with acetonitrile and 3:1 chloroform:methanol mixture. Metabolites of BBIT were identified using a combination of hybrid liquid chromatography/mass spectroscopy (LC-MS) systems and nuclear magnetic resonance (NMR). Radioactivity was measured using a Packard Tricarb liquid scintillation counter combined with a DEBRA computer program. Urinary metabolites were isolated from 300 mg [^{14}C]-BBIT/kg 12 hour samples.

4. **Statistics:** Although no statistical analysis was provided, results were reported as mean values with standard deviations and were considered appropriate.

5. **Pharmacokinetic Calculations:** Total radioactivity data were collected and calculated using a validated DEBRA 5.4.10.51 computerized data acquisition system. The limits of detection (LOD) for radioactivity were equal to twice the scintillation background rate as determined by the scintillation counts for the reagent blank assumed to be deionized water). For measurements below twice the background, the DEBRA computer system raised the scintillation count to equal twice the background value to derive a tissue LOD concentration expressed as µg equiv BBIT/g. The DEBRA computer system also calculated group mean tissue concentrations.

II. RESULTS:

A. PHARMACOKINETIC STUDIES:

1. **Preliminary experiment:** No preliminary study was performed.

2. **Absorption:** Following administration of a single oral dose of 5 mg or 300 mg [^{14}C]-BBIT/kg to male rats, peak concentrations of radioactivity were measured in the blood 1 hour and 4 hours post-dosing (T_{max}), respectively, indicating rapid and extensive absorption of the test substance. Peak concentrations (C_{max}) were 1.4 µg equivalents of BBIT/g of blood or

44.3 µg equivalents BBIT/g, respectively. Blood concentrations declined steadily and were below the limits of detection 24 hours following the 5 mg dose and within 48 hours of the 300 mg dose.

Table 4. Cumulative excretion of radioactivity in urine, cage wash, expired air, and feces of [^{14}C]-BBIT (mean distribution) for male rats from test groups C (rpt) and D[a, b]

	5 mg [^{14}C]-BBIT/kg			300 [^{14}C]-BBIT/kg		
Time point (hours)	Urine + Cage Wash (%)	Feces (%)	Urine + cage Wash + Feces (%)	Urine + Cage Wash (%)	Feces (%)	Urine + cage Wash + Feces (%)
0-12	58.53	NA	58.53	42.10	NA	42.10
0-24	74.05	18.34	92.39	67.37	11.90	79.27
0-48	79.53	19.93	99.46	75.77	14.32	90.09
0-72	NA	NA	NA	79.06	16.37	95.43

[a] Data obtained from Tables 4 and 6 of the study report located on pages 40 and 42

[b] N=2 rats/dose

Table 5. Cumulative excretion of radioactivity in urine, cage wash, expired air, and feces of [^{14}C]-BBIT (mean distribution) for male rats from test groups E and F

	5 mg [^{14}C]-BBIT/kg			300 [^{14}C]-BBIT/kg		
Time point (hours)	Urine + Cage Wash (%)	Feces (%)	Urine + cage Wash + Feces (%)	Urine + Cage Wash (%)	Feces (%)	Urine + cage Wash + Feces (%)
0-12	56.66	NA	56.66	42.76	NA	42.76
0-24	67.46	21.37	88.83	72.38	17.03	89.41
0-48	70.26	22.74	92.99	77.8	19.02	96.83
0-72	70.87	22.94	93.81	80.17	19.25	99.42

Data obtained from Tables 8 and 10 of the study report located on pages 44 and 46

3. **Tissue distribution:** Distribution of [^{14}C]-BBIT in rat tissues/organs is summarized in Table 6. The greatest concentrations of radioactivity from both the 5 mg/kg and 300 mg/kg doses were detected in the bladder and GI tract within one hour and 4 hours post-dosing, respectively. The significant amounts of radioactivity in these tissues were attributed to residues from urine and intestinal tract contents. Radioactive tissue concentrations declined rapidly and reached background levels within 48 hours for most tissues. Three days following oral administration of 5 mg [^{14}C]-BBIT /kg, total tissue and carcass residual radioactivity equaled 0.3% of the total administered dose. The highest radioactive residues three days post-dosing were seen in the lymph nodes, abdominal fat, pancreas, skin, residual carcass, liver, and kidney. Similar results were seen for the 300 mg [^{14}C]-BBIT/kg rats; however the residues for the kidney were below the LOD at 72 hours post-dosing.

Table 6: Distribution of radioactivity in rat tissues/organs after administration of [^{14}C]-BBIT[a] (Mean Concentrations)

	µg equivalents of BBIT/g[a, b, c]											
	5 mg [^{14}C]-BBIT/kg male rats dose level						300 mg [^{14}C]-BBIT/kg male rats level					
Tissue/ organ	**1 hr**	**4 hrs**	**12 hrs**	**24 hrs**	**48 hrs**	**72 hrs**	**4 hr**	**12 hrs**	**18 hrs**	**24 hrs**	**48 hrs**	**72 hrs**
Abdominal Fat	0.561	0.154	0.105	<0.009	<0.004	0.041	25.284	16.418	1.942	1.123	0.473	1.006
Adrenals	1.144	0.456	0.224	0.035	<0.011	<0.011	52.032	24.355	8.614	3.585	<0.071	<0.440
Bladder	32.702	27.270	4.103	0.331	0.040	<0.016	3031.488	1152.011	63.280	74.061	<4.648	2.749
Bone	0.452	0.201	0.118	0.017	<0.022	<0.010	17.446	12.414	3.193	1.946	<0.656	<0.875
Brain	0.356	0.148	0.076	0.011	<0.003	<0.003	18.855	12.184	3.428	1.846	<0.471	<0.366
Epididymis	0.970	0.377	0.374	0.033	0.004	<0.008	47.594	25.252	6.596	3.824	0.359	<0.288
Eyes	0.561	0.248	0.138	0.024	<0.011	<0.006	20.707	17.341	6.561	3.351	0.751	<0.291
G.I Tract + contents	26.423	26.306	6.174	1.252	<0.001	<0.022	1739.043	617.728	212.935	145.486	10.686	2.638
Heart	0.955	0.374	0.164	0.021	0.005	<0.004	28.505	14.513	5.517	2.960	<0.237	<0.157
Kidneys	4.108	1.900	0.500	0.066	0.022	0.006	56.369	34.583	11.828	4.701	0.623	<0.218
Liver	1.887	0.893	0.441	0.091	0.028	0.007	72.696	39.844	12.206	6.875	0.945	0.583
Lungs	0.676	0.351	0.163	0.019	0.005	<0.003	47.882	17.259	<3.108	3.111	<0.234	<0.473
Lymph nodes	2.831	0.425	0.141	0.007	0.005	0.056	49.733	28.163	6.687	<1.979	1.156	1.217
Muscle	1.252	0.267	0.136	0.014	<0.003	<0.003	24.254	12.888	2.897	2.323	<0.179	<0.217
Pancreas	1.001	0.390	0.168	0.025	<0.005	0.032	39.607	23.974	8.440	3.467	<0.454	0.869
Plasma	1.431	0.593	0.213	0.029	0.008	<0.005	55.865	35.099	10.364	5.058	0.367	<0.146
Prostate	7.417	1.392	1.266	0.089	<0.008	<0.016	437.554	87.650	17.992	5.062	<0.436	<0.370
Residual Carcass	0.832	0.479	0.188	0.119	0.025	0.013	41.261	21.749	5.353	40459	1.680	0.827
Salivary gland	0.594	0.226	0.105	0.015	<0.004	<0.143	23.330	12.612	2.932	<1.492	0.238	<0.143
Sciatic nerve	0.760	0.347	0.753	<0.034	<0.021	<0.022	37.240	19.280	6.979	4.046	<1.544	<1.045
Skin	0.861	0.450	0.247	0.040	0.031	0.017	34.101	21.677	9.759	5.238	2.451	0.860
Spinal cord	0.382	0.164	0.080	<0.012	<0.006	<0.005	21.842	12.086	4.508	1.937	<0.401	<0.765
Spleen	0.830	0.265	0.111	<0.019	0.004	<0.004	19.007	16.693	4.873	<1.483	<0.208	<0.182
Testes	0.602	0.307	0.189	0.022	<0.004	<0.003	21.085	17.640	5.353	2.593	<0.355	<0.204
Thymus	0.896	0.356	0.158	0.020	<0.006	<0.003	24.779	18.333	4.995	2.514	<0.370	<0.657
Thyroid	1.185[d]	0.628	0.595	0.059	<0.023	<0.067	59.961	32.923	14.943	11.424	<4.680	<2.074

[a] Data obtained from Tables 11-12 pages 47-48 in the study report (MRID 48349201).

[b] There were discrepancies in time intervals stated in the text Section 6.4 (page 25) and presented in Tables 11-12 (pages 47-48) in the study report (MRID 48349201).

[c] N=4

[d] Mean calculated using three animals

Terminal half lives of elimination from select tissues (calculated from test Groups G and H) are shown in Table 7. Terminal half life values for abdominal fat, lymph nodes and pancreas were calculated using values from Group G only since Group E rats were slightly elevated in these fatty tissues. Terminal half lives for these select tissues ranged from 7.4 to 38.9 days for the 5

mg dose and from 7.4 to 19.6 for the high dose. The study author cautioned that these values were estimates determined from very low tissue concentrations.

Table 7. Half lives of radioactivity of [^{14}C]-BBIT in tissues[c]		
	5 mg [^{14}C]-BBIT/kg	300 mg [^{14}C]-BBIT/kg
Tissue	Days	Days
Abdominal Fat	8.4[a]	15.8[b]
Kidneys	10.2	NA
Liver	13	9.5
Lymph nodes	8.5[a]	14.4[b]
Pancreas	7.4[a]	7.4[b]
Residual Carcass	14.8	19.6
Skin	38.9	18.4

[a] Tissue samples from Group G

[b] Tissue samples from Group H

[c] Data obtained from pages 49-50, Tables 13 and 14

4. **Excretion:** A summary of the excretion profile for orally administered [^{14}C]-BBIT /kg is shown in Table 8. The majority of the administered doses of [^{14}C]-BBIT /kg were excreted in the urine (65-78%) and feces (16-23%) with trace amounts detected in expired air. Total mean excretion was approximately 95% 72 hours following administration of 5 mg [^{14}C]-BBIT /kg (71% in urine and 23% in feces) and approximately 100% 72 hours following administration of 300 mg [^{14}C]-BBIT /kg (80% in urine and 19% in feces). Nearly all administered [^{14}C]-BBIT was excreted within 24 hours of dosing.

   The total mean recovery of administered radioactivity (defined as the summation of radioactivity detected in excreta, tissues, cage washings, and residual carcasses) was approximately 100% for the 5 mg /kg dose and slightly lower, 98%, for the 300 mg/kg dose.

Table 8. Mean percent recovery of radioactivity in tissues and excreta after administration of [^{14}C]-BBIT in male rats[a]				
	Test groups C and D[b]		Test groups E and F[c]	
	5 mg [^{14}C]-BBIT/kg:	300 mg [^{14}C]-BBIT/kg:	5 mg [^{14}C]-BBIT/kg:	300 mg [^{14}C]-BBIT/kg:
Expired air	0.03	0.06	NA	NA
GTI + contents	0.16	0.05	NA	NA
Carcass + blood + tissue	0.73	0.50	0.27	0.31
Cage wash	1.64	1.60	5.83	8.01
Urine	77.89	77.46	65.04	72.16
Feces	19.93	16.37	22.94	19.25
Total percent radioactivity recovered	100.39	96.03	98.08	99.73

[a] Data obtained from pages 39-46 in the study report (MRID 48349201).

[b] N=2

[c] N=4

NA = Not Applicable

B. METABOLITE CHARACTERIZATION STUDIES:

Metabolites of BBIT, summarized in Table 9, were identified and quantified using urine and fecal samples from rats orally exposed to single doses of 5 mg [^{14}C]-BBIT/kg or 300 mg[^{14}C]-BBIT/kg; insufficient samples were obtained from expired air for analysis (groups C, D, E, and F). Similar metabolites were identified from animals at both doses.

Table 9. Metabolic profile in excreta of rats dosed with [^{14}C]-BBIT[a]

	5 mg [^{14}C]-BBIT/kg body weight		300 mg [^{14}C]-BBIT/kg body Weight	
	Percent of administered dose		Percent of administered dose	
Compound	Urine (0-48 hour)	Feces (0-48 hour)	Urine (0-48 hour)	Feces (0-48 hour)
N-BBIT (Metabolite 9)	0.3	-	0.1	0.5
Metabolite 1 (N-dealkylated metabolite 5)	0.6	-	2.5	-
Metabolite 2 (dihydroxylated product of metabolite 8)	0.2	1.3	-	-
Metabolite 3 (dihydroxylated product of metabolite 8) isomer	0.6	0.6	1.3	-
Metabolite 4 (Unknown)	-	1.4	0.4	-
Metabolite 5 (methyl sulfoxide metabolite)	27.0	8.3	25.8	3.6
Metabolite 6 (Unknown)	0.3	3.4	0.3	1.2
Metabolite 7 (S-glucuronide)	23.6	5.4	22.6	12.4
Metabolite 8 methyl sulfoxide metabolite of parent compound)	-	-	0.1	0.2
Metabolite 10 (Unknown)	-	-	-	0.3
Metabolite 11 (methyl sulfoxide metabolite with carbonyl substitution	11.8	2.4	18.6	0.9
Total	64.4	22.8	71.7	19.1

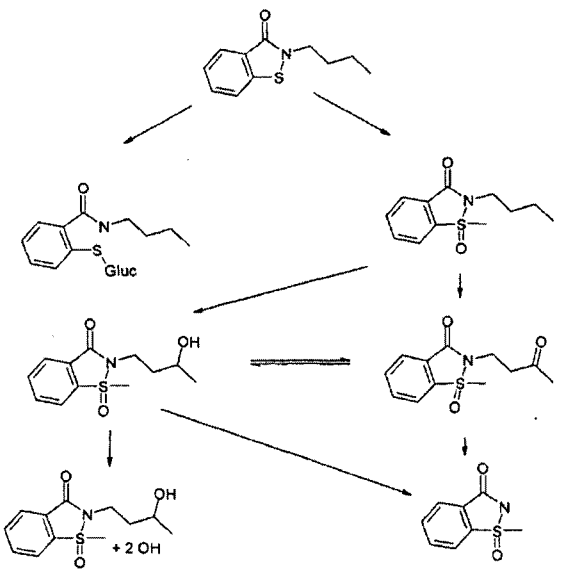
[a] Data obtained from pages 30-31 in the study report (MRID 42862203).

Un-metabolized BBIT, identified as metabolite 9, was detected in small amounts in all urine samples and in fecal samples from rats treated with 300 mg [^{14}C]-BBIT /kg. The two major metabolites identified in urine from rats dosed with 5 mg/kg and 300 mg/kg were metabolite 5 and metabolite 7. Metabolite 5 was identified as a methyl sulfoxide derivative of BBIT and accounted for approximately 26-27% of the respective administered doses. Metabolite 7, an S-glucoronide metabolite, accounted for approximately 23-24% of the administered doses. Both of these metabolites were also detected in the feces, although at lower and more variable concentrations. Metabolite 5 accounted for 8.3% of the 5 mg [^{14}C]-BBIT /kg dose but only 3.6% of the 300 mg/kg dose. Conversely, metabolite 7 was 5.4% of the administered 5 mg [^{14}C] BBIT /kg dose but 12.4% of the 300 mg/kg dose. Metabolite 11, a

methyl sulfoxide metabolite of BBIT with carbonyl substitution, was a major urinary and minor fecal metabolite present in both low and high dose samples comprising 11.8-18.6% of the administered doses detected in urine but only 0.9-2.4% in the feces. The remaining metabolites were detected in low concentrations.

The metabolism of BBIT is thought to occur via two major routes (Figure1) based on identification of three major metabolites identified in rats. Route 1 involves opening of the isothiazoline ring followed by conjugation of the sulfur atom with glucuronic acid. Route 2 involves direct oxidation and methylation of the sulfur atom in the isothiazoline rings forming the methyl sulphoxide which then undergoes further oxidation of the N-alkyl side chain and/or N-demethylation.

Figure 1. Proposed metabolic pathway for N-BBIT in the rat.



Source: Figure obtained from page 25 in the study report MRID 48262203.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS**: Following oral administration of 5 mg of [^{14}C]-BBIT to rats, T_{max} was 1 hour and C_{max} was 1.4 µg equivalents of BBIT/g of blood. The corresponding T_{max} and C_{max} values for the 300 mg [^{14}C]-BBIT /kg dose was 4 hours and 44.3 µg equivalents BBIT/g, respectively. Blood concentrations declined steadily and were below the LOD within 48 hours. [^{14}C]-BBIT was widely distributed and peak tissue radioactivity was measured 1 hour following the 5 mg/kg dose and 4 hours following the 300 mg/kg/dose. The greatest concentrations of radioactivity from both the 5 mg/kg and 300 mg/kg doses were detected in the bladder and GI tract and were attributed to residues from urine and intestinal tract contents. Radioactive tissue concentrations declined rapidly and reached background levels within 48 hours for most tissues. Similar totals and residual

radioactivity was 0.3% for both doses three days following oral administration of 5 mg [^{14}C]-BBIT/kg.

The majority of the administered doses of [^{14}C]-BBIT/kg were excreted in the urine (65-78%) and feces (16-23%) within 72 hours of dosing. Only trace amounts of radioactivity were detected in expired air.

Metabolites of [^{14}C]-BBIT were identified predominantly in the urine and feces with insignificant amounts being detected in exhaled air. [^{14}C] -BBIT undergoes essentially complete biotransformation following oral administration in the rat with only small amounts of un-metabolized test substance detected in the urine and feces. Qualitatively similar metabolites were detected in the urine and feces at both doses. A total of 11 metabolites were identified and quantified with only three being considered as major metabolic products. The three major metabolites were identified as a methyl sulfoxide derivative of BBIT (approximately 26-27% of the respective administered doses), an S-glucoronide metabolite (approximately 23-24% of the administered doses) and a methyl sulfoxide metabolite of BBIT with carbonyl substitution (11.8-18.6% of the administered doses detected in urine but only 0.9-2.4% in the feces). The study author proposed two metabolic pathways for [^{14}C]-BBIT. The first involves opening of the isothiazoline ring followed by conjugation of the sulfur atom with glucuronic acid, while the seconds involves direct oxidation and methylation of the sulfur atom in the isothiazoline rings forming the methyl sulphoxide which then undergoes further oxidation of the N-alkyl side chain and/or N-demethylation.

B. REVIEWER COMMENTS: Overall, our reviewers agree with the study authors' conclusions regarding the measured pharmacokinetic parameters for [^{14}C] BBIT which indicate that BBIT is rapidly absorbed and distributed following oral administration and primarily excreted in the urine with smaller percentages excreted in the feces. These findings are confirmed by the high concentrations of radioactivity measured in the bladder and GI tract. Total recoveries were greater than 90% which are considered acceptable according to OECD and OPPTS guidelines.

Two experimental deficiencies were identified. The study author did not report the age of the male rats used in this study. Specific housing assignments were not included in the study report. The study author notes that rat data from groups E and G were used for estimating terminal half-lives for selected tissues on page 27 of the study report. The study author also notes that group E rats were group housed for the experiment. However, group E rats were used for excretion studies and according to guidelines, are expected to be housed in individual metabolism cages. It is presumed that the study author intended to discuss group H rats rather than group E rats on page 27 (tissue depletion study group).

The reviewers note that adequate analytical methods were used to identify and quantify the metabolites of BBIT. The proposed metabolic pathways are reasonable given the identified metabolites.

C. STUDY DEFICIENCIES:

Minor study deficiencies include:

1. Age of male rats at study initiation not reported
2. Specific housing assignments not included in the study report
3. Unclear housing assignments for group E and group H rats
4. Discrepancies in time intervals for measurements of radioactivity in tissues stated in the text and listed in the tables

D. STUDY CLASSIFICATION: The metabolism study (MRID48349201) in the rat is classified as acceptable/guideline and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

The metabolism study (MRID 48262203) in the rat is classified as acceptable/guideline and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

Sign-off Date : 01/17/12
DP Barcode No. : D386070

TXR No. : 1,003,231

13544

R196985

Chemical Name: 1,2-Benzisothiazol-3(2H)-one, 2-butyl-

PC Code: 098951
HED File Code: 90110 AD RASSB Tox DERs
Memo Date: 1/17/2012
File ID: TX1003231
Accession #: 000-00-0137

HED Records Reference Center
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